SHALLOW PAN CULTIVATION TO ENHANCE THE YIELD OF BACTERIAL CELLULOSE

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ABSTRACT

Continuous filamentation of bacterial cellulose (BC) was successfully achieved by applying shallow incubation pan to regulate thickness of the BC gel produced by *Acetobacter xylinum*. Though the better yield of BC was shown by applying aerobic rotary cultibator than that by the static one, the yield of BC filament was enhanced remarkably by applying shallow pan cultivator with continuous wind up roller from the surface of the culture medium. The X-ray diffraction analysis and scanning electron microscopic observation of the BC filament showed that the filament was smooth and the fairly good orientation of BC molecules. The best average tensile strength was obtained for the filament prepared by hot alkaline treament and subsequent washing with distilled water followed by drying under tension. A carboxymetyl glucose (CM-Glc) residue was found in BC whose ion exchange capacity was enhanced remarkably only for lead ion, when *Acetobacter xylinum* was cultured in SH medium containing CM-Glc, CM-cellulose oligomer or CMC and glucose.

INTRODUCTION

The bacterial cellulose (BC) is well known as one of extracellular poly-saccharides to be produced by *Acetobacter xylinum*[1] in Schramm-Hestrin (SH) medium containing D-glucose (Glc) as a carbon source[2]. Because BC is a pure cellulose, it has attracted much attension in various manufacturing fields. In our previous studies on the preparation of of BC, we developed novel procedures to produce molecular variants of BC. Incorporation of aminosugar residues has been successfully attained by incubation of the bacteria that had been subcultured repeatedly in the medium containing *N*-acetylglicosamine (GlcNAc) and Glc or only GlcNAc as carbon source[3,4]. Under rotatory but not static conditions, a similar degree of GlcNAc incorporation was also observed when cultivation was carried out with air bubbling in the medium containing Glc and ammonium chloride[5].

Although much effort has been developed to the preparation and utilization of BC and its analogs, these methods and agents have not yet been incorporated as industrial resources, largely because of the high production costs involved. Given that complicated procedures are required for the preparation of fibers and films from these biosynthesized polysaccharides, a simple method, one that can serve to reduce these high production costs, has been requested.

In this paper, we describe the results of our investigations for such a simplified process, which was prompted by recent success with regard to surface polymerization of nylon capable of giving superpolymer films or filaments directly from the interface of organic and aqueous phases[6,7]. We have designed a shallow stainless pans[8-11] to regulate the gel thickness better during the incubation and physical properties of the resultant filaments were examined. An introduction of carboxymethyl glucose (CM-Glc) residue in BC was succeeded when *Acetobacter xylinum* was cultured in SH medium containing carboxymethyl cellulose (CMC) and glucose. The ion exchange capacity of BC containing CM-Glc residue was enhanced remarkably only for lead ion.

EXPERIMENTAL

Culture conditions

A wild type of *Acetobacter xylinum* ATCC 10245 strain was subcultured at 28 °C in Schramm-Hestrin (SH) mediumcontaining Glc as a carbon source, and repeatedly transferred to the new culture medium every 3 days. Shallow pans (100 mm or 200 mm of width, 400 mm of length and 7 mm of depth) were designed to prepare a thin BC gel first and then wind up to roller at the rate of 35 mm/h directly from cultivation medium as shown in Figure 1. The cultivation of *A. xylinum* was proceeded for a couple of days under static condition and thenthin gel formed on the surface of medium was picked up to wind up roller continuosly for a week. A separator was attached to prepare the strips of 30 mm width for the pan of 200 mm width. The whole apparatus was set in an incubator in which temperature was maintained at 28 °C and filtered air was passed through the incubator. For the incorporation of CM-Glc residue into BC, CMC was mixed into glucose SH medium on the cultivation of BC. The content of the CMC in SH medium was 0.5%(w/v).

Purification of filament

The wound filament was boiled for 3 h in 2% SDS solution, washed with distilled water, and boiled again in the 4% aqueous sodium hydroxide solution for 1.5 h. Three samples of the filaments were prepared by washing of the resulting filament with distilled water (W), with 10 % aqueous ethylene glycol (E), or with both 10 % aqueous ethylene glycol and distilled water (WE). All filaments were subjected to air-drying under tension at less than 60 °C.

Measurements

The stress-strain diagrams of the filaments were obtained using a Shimadzu Auto Graph AGS-500D apparatus at a guide distance of 25 mm. Wide-angle X-ray diffraction patterns were recorded by using a MAC M18XHF X-ray diffractometer. The X-rays were generated at 40 kV and 100 mA using nickel-filtered Cu*K*a radiation.

RESULTS AND DISCUSSION

To make thinner BC gel suitable for direct and continuous filamentation, we devised a shallow pan for the incubation of *A. xylinum*. In a preliminary incubation under static conditions, we found that thin BC gel was obtained on the surface of the culture medium, and that the gel were strong and elastic enough to pick up and manipulate. One side of the shallow pan was curved gently to permit harvest of the pellicle through a narrow mouth. The yield of the BC was enhanced using shallow pan method (4.1 - 5.6 %) compared with static (1.6 %) and rotatory (2.7 %) methods[10,11]. The thin BC gel was to be directly passed through a bath containing aqueous SDS solution to reduce the bacterial activity and then the filament would be wound slowly on an attached basket. Produced fibrous gel was streched under slightly twisted mode. The shallow pan would be also effective in conserving the total amount of culture medium used for the incubation. As the filament was too thick to prepare filament in the case of wider pan (200mm width), a separator was attached to prepare the strips of 30mm width. The separator was so effective to prepare thinner filament. Tretments of the BC fiber was easy because the obtained fibrous BC was collected wound with bobin. In addition, winding by a wide roller has the possibility to prepare the BC film.

A SEM image of BC filament showed that the BC filament with normal water-washing (Filament W) has good alignment cellulose moieties and slightly twisted fiber mode. The cut surface of filament shows the melting of the inner part of filament to form a fiber bundle. The X-ray diffraction pattern suggested that Filament W has a higher orientation of molecules than either Filament E or WE. This finding is consistent with those from SEM observation.

The tensile properties and the stress-strain curves for the filaments obtained by different washing procedures (Filaments W, E, and WE) are summarized in Table 1 and Figure 2, respectively. The stress-strain diagram shows that Filament WE has poor tensile strength. Deniers of ethylene glycol-treated filaments (Filament E) tended to become double compared with the filament prepared by normal water-washing (Filament W), a finding that might reflect the slightly disturbed orientation of molecules in the filaments by the trapping of ethylene glycol in the network. Among these three kinds of filaments, normal water-washing filament (Filament W) had good mechanical strength; the best sample revealed 6.1 g denier^{-1} for the tensile strength and 106 g denier^{-1} for Young's module and their average values were 4.4 g denier^{-1} and 90.4 g denier^{-1}, respectively. They were significantly stronger than the original BC filament and were comparable to cotton (3.0 - 4.9 g denier^{-1} for the tensile strength and 68 - 93 g denier^{-1} for Young's module[12] and the other fibers[13]. The filaments prepared by ethylene glycol treatment (Filaments E and WE) seem less suitable for practical use. The low tensile strength and Young's modulus obtained by aqueous ethylene glycol treatment after cleaning of the filaments may be due to obstruction of molecular orientation followed by incomplete crystallization of cellulose molecules.

The CM-Glc residue was incorporated using the SH medium containing CMC. Around 10% of residual incorporation of CM-Glc was the maximum at 0.5%(w/v) of CMC mixing in the SH medium. Produced CM-BC was applied to investigate the ion exchange properties following to extensive washing with SDS and 4% sodium hydroxide aqueous solutions at boiling temperature, respectively. A significant increase of adsorption for lead ion was observed on the CM-BC as shown in Figure 3 comparing with ion exchange profile of original CMC.

CONCLUSIONS

Continuous filamentation using a shallow pan for the incubation was found to be useful for the regulation of the thickness of the BC gel. The yield of the BC was enhanced remarkably by applying this method compared with static and rotatory methods. The tensile

strength of the filament was found to be significantly stronger than the ordinary cellulose fibers and a good orientation of molecules was shown by both the X-ray diffraction pattern and SEM observation. Around 10% of residual incorporation of CM-Glc was succeessfully performed using CMC containing SH medium. A significant increase of absorption for lead ion was observed on the CM-BC comparing with ion exchange profile of original CMC.

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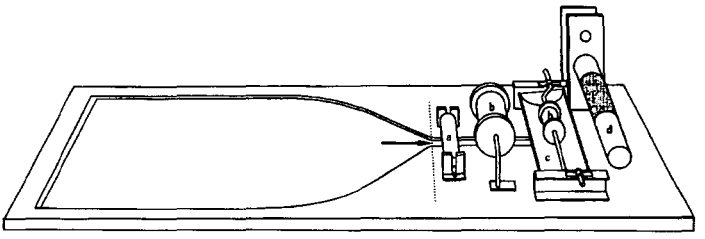

Fig. 1 Outline of culture pan for the direct filamentation of BC. a, sinker; b, roller; c, washing pan; d, wind up roller

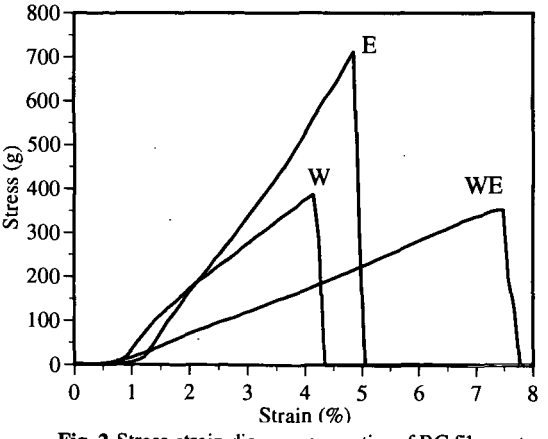

Fig. 2 Stress-strain diagram properties of BC filaments.

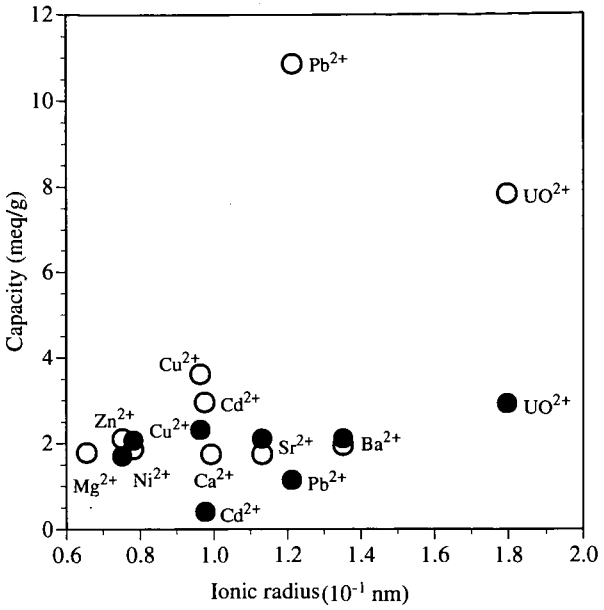

Fig. 3 Metal ion adsorption profile of CM-BC (O) and that of original CMC (●).

Table 1 Tensile Properties of BC Filament

Sample[a]	Elongatior (%)	Size (denier	Tensile stres (g/denier)	Tensile strengtl (g/denier)	Young's modulu (g/denier)
W1	4.2	108.0	267.4	3.9	93.8
W2	6.0	169.2	223.3	5.6	93.6
W3	4.5	108.0	431.5	3.6	80.5
W4	3.8	108.0	242.4	3.0	78.9
W5	5.8	140.4	386.4	6.1	105.4
E1	5.2	216.0	310.2	3.6	67.9
E2	3.7	216.0	386.4	3.5	93.7
E3	3.8	216.0	284.7	3.1	83.1
WE1	7.7	180.0	293.6	2.1	27.3
WE2	3.7	180.0	249.3	1.9	52.5
WE3	4.2	180.0	341.3	3.1	74.8

a) Obtained filament was rinsed extensively with water (W), with 10% aqueous ethylene glycol (E), or successively with 10% ethylene glycol and water (WE). All filaments were air-dried under tension.